椿叶花椒果实精油对两种蚊虫的 生物活性及成分分析

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摘要:为筛选环保型的植物源灭蚊活性物质,研究了椿叶花椒 Zanthoxylum ailanthoides 果实精油对白纹伊蚊 Aedes albopictus 和致倦库蚊 Culex pipiens quinquefasciatus 成蚊的熏杀活性,并测试了精油对这两种蚊幼虫的毒杀效果。此外,采用 GC-MS 分析了该精油的化学成分。结果表明:在 $102.145~\mu g\cdot cm^{-3}$ 熏杀剂量下,精油对白纹伊蚊和致倦库蚊成蚊的 KT_{50} 值分别为2.788和 3.505 min,24 h 致死率分别为 100%~n 97.53%。该精油对白纹伊蚊和致倦库蚊 4 龄幼虫的 LC_{50} 值分别为 45.237 和 20.759 mg· L^{-1} 。从椿叶花椒果实精油中共鉴定出 14 种化合物,其中 2-十一酮为主要成分,相对含量为89.86%。结果说明椿叶花椒果实精油对两种蚊虫均有明显的杀虫活性,具有开发成植物源灭蚊剂的潜力。 **关键词**:椿叶花椒;精油;杀虫活性;白纹伊蚊;致倦库蚊;有效成分

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Bioactivity and components of essential oil from Zanthoxylum ailanthoides fructification against two mosquito species

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Abstract: To find some eco-friendly components from plants as mosquitocide, the fumigant activity of essential oil from Zanthoxylum ailanthoides fructification to the adults of Aedes albopictus and Culex pipiens quinquefasciatus was tested, and its insecticidal activity against the larvae and pupae of the two mosquito species was evaluated. The volatile components of this oil were also analyzed by gas chromatography-mass spectrometry. The results showed that at the dosage of 102.145 μg · cm⁻³, the KT₅₀ values of this oil for adults of Ae. albopictus and Cx. pipiens quinquefasciatus were 2.788 and 3.505 min, respectively, while the 24 h mortalities of adults of the two mosquito species were 100% and 97.53%, respectively. The 24 h LC₅₀ values of this oil for 4th instar larvae of Ae. albopictus and Cx. pipiens quinquefasciatus were 45.237 and 20.759 mg · L⁻¹, respectively. Fourteen chemical compounds were identified from the essential oil from Z. ailanthoides fructification, and the main component of this essential oil is 2-undecanone with the peak-area ratio of 89.86%. The results demonstrate that the oil from Z. ailanthoides fructification has strong insecticidal activity against A. albopictus and Cx. pipiens quinquefasciatus, and it has a great potential to be developed as a natural mosquitocide.

Key words: Zanthoxylum ailanthoides; essential oil; insecticidal activity; Aedes albopictus; Culex pipiens quinquefasciatus; active ingredients

蚊虫可传播多种疾病(如登革热、疟疾、丝虫病等)。蚊虫控制研究在预防医学中始终是一个重要的科研课题。长期以来化学杀虫剂是有效控制蚊虫的主要手段,但大量频繁地施用化学杀虫剂使蚊虫

抗药性日益增强,且对环境造成巨大压力,研发性能优良的环保型植物源灭蚊剂刻不容缓(崔峰和乔传令,2007;彭映辉等,2007)。目前,国内外的研究者们已发现多种植物的精油对蚊虫具有驱灭活性

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(Vahitha et al., 2002; Prabakar and Jebanesan, 2004; Rajkumar and Jebanesan, 2004; Amer and Mehlhorn, 2006; 郝蕙玲等, 2006; 周利娟等, 2006; Gleiser and Zygadlo, 2007; 王桂清等, 2007; Chapagain et al., 2008; Cheng et al., 2008, 2009b; Autran et al., 2009; 彭映辉等, 2009), 但尚无椿叶花椒 Zanthoxylum ailanthoides 精油对蚊虫杀灭活性的研究报道。椿叶花椒是芸香科(Rutaceae) 花椒属落叶乔木, 在我国长江以南地区均有分布,资源十分丰富。本研究结果表明椿叶花椒果实精油对白纹伊蚊和致倦库蚊的成蚊有明显的熏杀活性,对两种蚊虫各龄期幼虫及蛹有显著的毒杀活性。

1 材料与方法

1.1 供试植物及蚊虫

椿叶花椒 Z. ailanthoides 新鲜成熟果实于 2007 年 11 月 15 日采自湖南省森林植物园。

白纹伊蚊 Ae. albopictus 和致倦库蚊 Cx. pipiens quinquefasciatus 是国家标准卫生用杀虫剂药效评价昆虫,种源由湖南省疾病预防控制中心提供。饲养温度 $26 \pm 2^{\circ}$ 、相对湿度为 $70\% \pm 5\%$,光周期14L: 10D。

1.2 椿叶花椒果实精油的制备

采用水蒸汽蒸馏装置,将用蒸馏水洗净后的 100~g 椿叶花椒新鲜成熟果实,装入 1~000~mL 圆底烧瓶中,加入 700~mL 蒸馏水,水蒸汽蒸馏 5~h,收集精油于棕色细口试剂瓶中,加入适量的无水硫酸钠干燥过夜,冷藏于 4° C 的冰箱中备用;精油提取率(油/鲜重)为 1.20%,精油密度为 $0.881~g \cdot mL^{-1}$ 。

1.3 椿叶花椒果实精油对白纹伊蚊和致倦库蚊成 蚊的熏杀活性测定

参照杨频等(2004)的方法(三角瓶密闭熏蒸法)和中华人民共和国国家标准《农药登记卫生用杀虫剂室内药效试验方法——电热片蚊香的室内药效测定方法 GB 13917.5-1992》进行优化测试,在常温下测定椿叶花椒果实精油对白纹伊蚊和致倦库蚊成蚊的熏杀活性。在250 mL 三角瓶(实测容积为345 mL)内接入20 头羽化后2-3 d 未吸血的雌成蚊,并以白纱布封口,在白纱布上固定一块滤纸条(1 cm × 5 cm),在滤纸条上分别滴加5组不同熏杀剂量的精油,迅速用保鲜膜封口并开始计时,每2 min 观察记录一次试虫的击倒数,持续熏蒸20 min 后将全部试虫移入洁净的1000 mL 大烧杯中,并用白纱布封口;用含有5% 葡萄糖水的海绵球饲

养,饲养 24 h 后观察记录试虫死亡数,每个处理组重复 5 次,同时设空白对照。测定条件:温度 26 ± 1 ℃,相对湿度 60% ± 5%。

1.4 椿叶花椒果实精油对白纹伊蚊和致倦库蚊幼 虫的毒杀活性测定

参照 Nathan 等(2006)的方法(浸液法)进行优化测试:将椿叶花椒果实精油充分溶解于无水乙醇中(分析纯,纯度≥99.7%),用蒸馏水稀释到5个浓度。在50 mL 锥形瓶中分别接入20头供试蚊虫(白纹伊蚊和致倦库蚊1~4龄幼虫及蛹),将25 mL上述各浓度组的精油溶液分别加入到锥形瓶中;同时以5个不同浓度组的印楝素(azadirachtin)作为对照药剂处理两种蚊虫各龄期幼虫及蛹。每组处理设5次重复,并计算出各组精油溶液中乙醇的浓度,配制等浓度的乙醇溶液用作空白对照。试验期间不喂食,处理24 h 后记录幼虫与蛹的死亡数目。测定条件:温度26±1℃,相对湿度60%±5%。

1.5 椿叶花椒果实精油化学成分分析

GC-MS 为 Agilent 6890GC-5973, 采用 HP-5 弹性石英毛细管柱(30 m×320 μ m×0.25 μ m)(美国安捷伦公司)。进样量为 1 μ L, 升温程序为:柱温 40℃保持 8 min, 2℃ · min $^{-1}$ 程序升温至 100℃,保持 2 min 后,3℃ · min $^{-1}$ 程序升温至 250℃ 保持 5 min;进样口温度为 250℃。高纯氦气作为载气,流量 60 mL · min $^{-1}$ 。离子源为 EI,电离电压为 70 eV,离子源温度 220℃,扫描质荷比范围:35~500 amu。采用 NIST 98 数据库结合以往文献确定 椿叶花椒果实精油的化学成分。

1.6 数据处理

毒杀活性测定结果和熏杀活性测定结果均采用概率值分析方法,用 SPSS 13.0 统计软件分别计算出 LC_{50} 值和 LC_{95} 值、 KT_{50} 值和 KT_{95} 值、95% 置信区间和毒力回归方程,并对毒力回归方程进行卡方检验 (χ^2) (显著水平为 $\alpha=0.05$ 和 $\alpha=0.01$)。毒杀和熏杀试验中,24 h 时的校正死亡率根据以下公式求得:

校正死亡率 =

<u>处理组死亡率 - 对照组死亡率</u> × 100% 1 - 对照组死亡率

2 结果与分析

2.1 椿叶花椒果实精油对白纹伊蚊和致倦库蚊成 蚊的熏杀活性

试验结果表明, 在短时间(20 min)的持续熏蒸

下,5 组不同剂量的椿叶花椒果实精油对白纹伊蚊和致倦库蚊成蚊的熏蒸活性有差异;随着精油剂量的增加,试虫的击倒速度明显加快,24 h后的死亡率明显上升。在102.145 µg·cm⁻³的熏蒸剂量下,精油对白纹伊蚊和致倦库蚊成蚊的 KT₅₀值分别为

2.788 和 3.505 min。在 5 个不同剂量的处理组中,精油对白纹伊蚊的击倒速度均快于致倦库蚊;且 24 h后,白纹伊蚊的死亡率均高于致倦库蚊;空白对照组无死亡(表 1)。

表 1 椿叶花椒果实精油对白纹伊蚊和致倦库蚊成蚊的熏蒸效果

Table 1 Fumigation effect of essential oil from Zanthoxylum ailanthoides fructification against adults of Aedes albopictus and Culex pipiens quinquefasciatus

试虫 Tested colonies	剂量 Dosage (µg・cm ⁻³)	毒力回归方程 Regression equation	KT ₅₀ (KT ₉₅) (min)		信区间 idence limit UCL KT ₅₀ (KT ₉₅) (min)	- 斜率 Slope	卡方值 (χ^2)	24 h 时校正死 亡率 Corrected mortality (%)
 白纹伊蚊	6.384	Y = -7.283 + 7.476X	9.423(15.639)	8.576(13.977)	10.224(18.539)	0.973	1.933 **	19.97
Ae. albopictus	12.768	Y = -6.032 + 7.494X	6.382(10.580)	5.710(9.367)	7.011(12.776)	1.061	0.782 **	62.33
	25.536	Y = -5.326 + 7.154X	5.554(9.430)	4.883(8.246)	6.165(11.728)	1.111	1.457 **	76.24
	51.072	Y = -4.201 + 6.313X	4.628(8.433)	3.995(7.263)	5.217(10.672)	0.947	3. 149 **	91.40
	102.145	Y = -3.026 + 6.795X	2.788(4.868)	2.354(4.063)	3.229(6.730)	1.246	0.374*	100
致倦库蚊	6.384	Y = -7.850 + 7.319X	11.821(19.834)	10.888(17.671)	12.742(23.711)	0.941	0.734 **	8.47
Cx. pipiens	12.768	Y = -7.858 + 7.623X	10.737(17.647)	9.879(15.812)	11.579(20.824)	0.954	6.538 **	25.02
quinquefasciatus	25.536	Y = -6.556 + 7.334X	7.833(13.128)	7.083(11.718)	8.536(15.553)	0.945	0.989 **	37.71
	51.072	Y = -5.018 + 6.216X	6.417(11.802)	5.674(10.368)	7.102(14.300)	0.805	0.642 **	56.42
	102.145	Y = -2.565 + 4.708X	3.505(7.834)	2.904(6.539)	4.070(10.276)	0.655	2.999*	97.53

^{*}表示方程的显著水平为 $\alpha=0.05$ Significant at P<0.05 level; **表示方程的显著水平为 $\alpha=0.01$ Significant at P<0.01 level. 下同 The same below.

表 2 椿叶花椒果实精油对白纹伊蚊和致倦库蚊成蚊的毒杀活性
Table 2 Toxicity of essential oil from Zanthoxylum ailanthoides fructification against adults
of Aedes albopictus and Culex pipiens quinquefasciatus

			95% 置	- 세호 Cl	卡方值(χ²)	
试虫	毒力回归方程	$\frac{\mathrm{LC}_{50}(\mathrm{LC}_{95})}{(\mathrm{\mu g}\cdot\mathrm{cm}^{-3})}$	95% Confidence limit			
Tested colonies	Regression equation		$LCL\ LC_{50}(\ LC_{95})$	$\mathrm{UCL}\;\mathrm{LC}_{50}(\mathrm{LC}_{95})$	- 斜率 Slope	Chi-square value
			(µg⋅cm ⁻³)	(µg⋅cm ⁻³)		
白纹伊蚊 Ae. albopictus	Y = -2.643 + 2.448X	12.009(56.402)	8.146(37.492)	15.940(123.823)	0.459	1.793*
致倦库蚊 Cx. pipiens quinquefasciatus	Y = -3.295 + 2.222X	30.401(167.174)	22.713(100.278)	41.558(435.253)	0.377	4. 432 **

椿叶花椒果实精油对白纹伊蚊和致倦库蚊的成蚊都具有明显的快速致死作用。24 h 后,白纹伊蚊和致倦库蚊成蚊的 LC_{50} 值分别为 12.009 和30.401 μ g·cm⁻³(表 2)。椿叶花椒果实精油对白纹伊蚊的 KT_{50} 值和 LC_{50} 值均比对致倦库蚊的低,说明精油对白纹伊蚊成蚊的熏蒸效果和毒杀活性都强于致倦库蚊。

2.2 椿叶花椒果实精油对白纹伊蚊和致倦库蚊幼 虫及蛹的毒杀活性

试验结果表明, 椿叶花椒果实精油对白纹伊蚊

和致倦库蚊各龄期幼虫及蛹具有高效、快速的致死作用,但毒杀活性有差异;空白对照组无死亡(表3)。24 h后,椿叶花椒果实精油对白纹伊蚊1龄幼虫的 LC₅₀值低于印楝素处理组;而精油对白纹伊蚊2~4龄幼虫和蛹的 LC₅₀值均与印楝素处理组的相近。椿叶花椒果实精油对致倦库蚊1~4龄幼虫的LC₅₀值均低于印楝素的处理组;而精油对致倦库蚊蛹的 LC₅₀值和印楝素处理组的相近。同时,试验结果表明,白纹伊蚊各龄期幼虫和蛹对椿叶花椒果实精油的耐受性比致倦库蚊强。

表 3 椿叶花椒果实精油对白纹伊蚊和致倦库蚊不同龄期幼虫和蛹的毒杀活性
Table 3 Toxicity of essential oil from Zanthoxylum ailanthoides fructification against larvae and pupae of

Aedes albopictus and Culex pipiens quinquefasciatus

试虫 Tested colonies	处理 Treatment	发育阶段 Developmental stage	毒力回归方程 Regression equation	LC ₅₀ (LC ₉₅) (mg · L ⁻¹)		置信区间 idence limit UCL LC ₅₀ (LC ₉₅) (mg·L ⁻¹)	Slope	卡方值(χ^2) Chi-square value
 白纹伊蚊	 椿叶花椒	1龄1st instar	Y = -6.758 + 8.124X	6. 795 (10. 824)	6. 066(9. 146)	7. 646(15. 825)	1. 762	0. 287 *
Ae.	果实精油	2龄2nd instar	Y = -4.669 + 3.177X	29. 487 (77. 802)	23. 065 (66. 040)	36. 572 (105. 256)	0. 461	0. 556 *
albopictus	Essential oil of	3龄3rd instar	Y = -8.568 + 5.392X	38. 819(78. 360)	31. 973 (64. 298)	45. 319(110. 835)	0. 946	0. 172 *
•	Z. ailanthoides	4龄4th instar	Y = -11.563 + 6.985X	45. 237 (97. 142)	38. 738(72. 148)	51. 358 (156. 545)	1. 300	1. 835 **
	fructification	蛹 Pupa	Y = -7.841 + 3.669X	137. 128(384. 993)	107. 842 (275. 744)	175. 954(694. 809)	0. 613	1. 438 ***
	印楝素	1龄1st instar	Y = -3.830 + 3.602X	11.567(33.102)	9. 201 (24. 150)	14. 530(56. 912)	0. 574	0. 398 *
	Azadirachtin ^a	2龄2nd instar	Y = -4.340 + 3.254X	21.554(69.022)	16. 920 (49. 286)	27. 532 (122. 473)	0. 508	0. 841 *
		3龄3rd instar	Y = -4.586 + 2.993X	34. 086 (120. 847)	26. 582 (84. 884)	43.677(215.993)	0.439	0. 608 *
		4龄4th instar	Y = -5.251 + 3.212X	43. 123 (140. 203)	33.765 (99.965)	54, 773 (248, 838)	0.499	0. 795 *
		蛹 Pupa	Y = -5.243 + 2.992X	56. 515 (200. 404)	43. 976 (140. 191)	72. 485 (364. 696)	0.452	1. 103 *
倦库蚊	椿叶花椒	1龄1st instar	Y = -3.275 + 4.515X	5. 314(12. 296)	4. 342(9. 196)	6.593(21.593)	0. 843	0.723 *
Cx. pipiens	果实精油	2龄2nd instar	Y = -3.568 + 3.456X	10.777(32.245)	8.347(22.691)	13.909(61.706)	0.610	0.570 *
quinque-	Essential oil of	3龄3rd instar	Y = -4.817 + 3.776X	18. 856(49. 344)	14. 956(37. 121)	23.645(82.806)	0.613	2.739 *
fasciatus	Z. ailanthoides	4龄4th instar	Y = -5.762 + 4.374X	20.759(51.406)	16.536 (37.891)	25. 855 (86. 947)	0.780	1. 837 *
	fructification	蛹 Pupa	Y = -10.019 + 4.978X	102.949(220.317)	83.750(165.361)	129. 736(384. 116)	0. 944	0. 368 *
	印楝素	1龄1st instar	Y = -3.456 + 3.612X	8. 891 (25. 155)	7. 054(18. 351)	11. 146(43. 988)	0.6073	1. 368 *
	Azadirachtin ^a	2龄2nd instar	Y = -5.100 + 3.514X	28. 260(83. 023)	22. 412 (60. 321)	35. 556(143. 579)	0. 557	0. 741 *
		3龄3rd instar	Y = -3.912 + 2.547X	34. 376 (152. 110)	26. 218 (102. 642)	44. 946 (286. 664)	0.357	0.832 *
		4龄4th instar	Y = -5.155 + 2.791X	70. 289 (273. 007)	50.401(184.662)	91.687(529.562)	0.422	2. 289 **
		蛹 Pupa	Y = -5.645 + 2.956X	81. 265 (292. 690)	63.415(200.456)	105.458 (559.399)	0.455	1. 760 **

a 阳性对照 Positive control.

2.3 椿叶花椒果实精油的化学成分

对椿叶花椒果实精油进行 GC-MS 分析, 共鉴 定出 14 种化合物, 检出物占精油挥发性成分总量 的 97. 30%, 其中萜烯类 8 种, 占挥发性成分总量的 3.91%;精油的主要化学成份是 2-undecanone(2-十一酮, 相对含量 89. 86%)(表 4)。

表 4 椿叶花椒果实精油化学成分

Table 4 Chemical compositions of essential oil from Zanthoxylum ailanthoides fructification

序号	保留时间(min)	化合物	分子式	分子量	相对含量(%) Relative
No.	Retention time	Compounds	M. F.	M. W.	content
1	14.403	1R-α-蒎烯 1R-alpha-pinene	$C_{10}H_{16}$	136	0.31
2	17.488	桧烯 Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{16}$	136	1.12
3	20.841	萜品油烯 Cyclohexene, 3-methyl-6-(1-methylethylidene)-	$C_{10}H_{16}$	136	0.12
4	21.739	D-柠檬烯 D-limonene	$C_{10}H_{16}$	136	0.43
5	24. 148	松油烯1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	136	0.32
6	26.986	2-壬酮 2-Nonanone	$C_9H_{18}O$	142	1.48
7	27.495	3-蒈烯 Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-, (1S)-(1S)-(+)-	$C_{10}H_{16}$	136	0.42
8	32.840	4-萜烯醇 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	$\mathrm{C_{10}H_{18}O}$	154	0.97
9	41.858	2-十一酮 2-Undecanone	$\mathrm{C}_{11}\mathrm{H}_{22}\mathrm{O}$	170	89.86
10	42.012	2-十二酮 2-Dodecanone	$\mathrm{C}_{12}\mathrm{H}_{24}\mathrm{O}$	184	0.05
11	48.781	金合欢烯 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	$C_{15}H_{24}$	204	0.29
12	51.688	癸酸乙烯 Vinyl decanoate	$\rm C_{12}H_{22}O_2$	198	0.90
13	51.882	正十八烷 Octadecane	$C_{18}H_{38}$	254	0.06
14	52.901	2-十三酮 2-Tridecanone	$\mathrm{C_{13}H_{26}O}$	198	0.97

3 讨论

本研究所用的对照药剂印楝素是目前世界上公认的理想的植物源杀虫剂,对 400 余种昆虫表现出不同的生物活性,具有广谱、对天敌干扰少、无明显的脊椎动物毒性和植物药害、在环境中降解迅速等优点。椿叶花椒果实精油对白纹伊蚊各龄期幼虫及蛹的毒杀效果与印楝素的毒杀效果相近,而对致倦库蚊各龄期幼虫的毒杀效果优于印楝素;同时,精油对两种成蚊也表现出明显的熏杀活性。椿叶花椒是一种有巨大开发潜力的森林植物资源,在我国分布广,资源丰富,其果实精油作为经济环保型植物源灭蚊剂具有一定的开发前景。

本文结果表明椿叶花椒果实精油中2-十一酮 的相对含量高达89.86%。据报道,2-十一酮是很 好的杀蛭剂、杀线虫剂和杀蠕虫剂,具有很强的生 物活性,能与昆虫神经细胞膜受体结合,改变离子 通道和生物学活性位点,可使昆虫神经传导受抑 制,起到毒杀作用(郝乃斌和戈巧英,1999;周天 等, 2006; Murugan et al., 2007)。 Tripathi 等(2004) 的研究结果表明 D-柠檬烯对斯氏按蚊 Anopheles stephensi 具有毒杀和驱避活性; Traboulsi 等(2005) 的研究发现 1R-α-蒎烯对骚扰库蚊 Culex pipiens molestus 具有毒杀活性;Odalo 等(2005)和宋湛谦等 (2006)的研究结果表明(1S)-(+)-3-蒈烯对蚊虫 具有驱避活性;Jaenson 等(2006)的研究发现松油烯 对蚊虫具有驱避活性;Cheng 等(2009a)的研究发现 金合欢烯对蚊虫具有毒杀活性。椿叶花椒果实精油 的化学成分中均含有上述6种化合物,由此可见, 椿叶花椒果实精油对两种蚊虫的毒杀活性很强,与 这些化合物的存在有着紧密的联系。目前,工业上 以冰乙酸和癸酸为原料,以浮石负载氧化镁为催化 剂,利用气固催化法来制备2-十一酮,摩尔产率为 75%。段建利等(2005)通过对载体和催化剂活性 组分的研究对 2-十一酮的人工合成途径进行改进, 以硅胶为载体, 以氧化镁为主组分的多组分催化剂 来制备 2-十一酮,可将其摩尔产率提高到 87.2%。 在进一步的研究中, 有必要将椿叶花椒果实精油中 的主要活性单体化合物 2-十一酮分离提纯, 研究其 对白纹伊蚊和致倦库蚊的毒杀活性和毒杀机理;同 时优化2-十一酮的人工合成途径,提高摩尔产率, 并减少生产过程对环境的污染,为研制高效、低毒、 无公害的环保型灭蚊剂提供科学依据和技术保障。

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